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The Grafting of Shortleaf and Other Pine Species

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A cleft graft of shortleaf pine made in 1947. Scion was taken from a typical littleleaf tree. From the start, new growth lacked any symptoms of littleleaf, and the tree, now 9 years old, continues healthy and vigorous.

THE GRAFTING OF SHORTLEAF AND OTHER PINE SPECIES

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Current interest in grafting southern pines stems from the rather recent development of forest tree improvement and genetics programs in the South. Methods of asexual propagation, such as rooting and grafting, are important tools in carrying out these programs because they enable the investigator to take advantage of many time-saving shortcuts.

The work described here is a continuation of that reported by Jackson and Zak (3) dealing with methods of grafting shortleaf pine (*Pinus echinata* Mill.). Methods used in the previous studies were developed for virus transmission tests when the causal factors of the littleleaf disease were yet unknown. A large number of grafts were made, including bark patch and approach grafts on both stems and roots. Scion material was taken from healthy as well as diseased trees. Stock trees included seedlings, saplings, and adult shortleaf pines.

Upon the discovery that a root fungus was mainly responsible for littleleaf (1), efforts were directed toward the selection and breeding of disease-resistant strains of shortleaf pine (9). The experience gained from the previous studies proved valuable for this work, and various methods of grafting are now being used to propagate selected shortleaf pines. Clones of this species are being planted in a central area for later use in intensive breeding studies and for the pilot development of seed orchards.

Recent work in the South has shown that grafting other southern pines is also feasible for experimental use in genetics and for the large-scale establishment of seed orchards. Zobel (10) successfully grafted scions of *Pinus taeda* L. from trees of different wood densities onto the same stock seedling. Bottle and side graft methods have been successfully employed by Chase and Galle (2) in the nursery grafting of *P. taeda*. They also obtained good results in the greenhouse grafting of this species and of *P. echinata*. Mergen (4) had good success grafting *P. elliottii* Engelm. using succulent tissue. In another study (5), he successfully grafted scions from young succulent slash pine seedlings onto stock of corresponding age of other species and other genera, in an attempt to induce early flowering. Perry (7) grafted *P. elliottii* enclosing the graft in a polyethylene bag.

METHODS IN GENERAL

Grafting was done in both the nursery and the greenhouse. Potted 1- or 2-year-old seedlings were commonly employed as stock plants. Some use, however, was made of trees in situ at the nursery. Every effort was made to have these stock trees in good vigor prior to grafting. High humidity was maintained around the entire grafted tree or the scion shoot for 3 to 8 weeks after grafting. In the greenhouse this was accomplished by an automatic humidity system. For outdoor grafts at the nursery, trees were fitted with individual humidity chambers.

Scion material was, if possible, collected shortly before grafting. Otherwise, the shoots were placed in the refrigerator at 4° or 5°C. packed in moist sphagnum moss, and removed as needed. Scion material was often held for 2 or 3 weeks in this manner.

Practically all shoots from adult trees were taken from the upper one-third of the crown, which bore the most vigorous material. No attempt was made to discriminate against shoots with male and female flower buds. These, and 1-year-old cones, however, were removed during grafting in later work. A lower degree of success was observed when fruiting structures were left attached--probably because stored foods became depleted in the shoot. Perry (7), however, considers it feasible to graft flower or cone-bearing twigs. In fact, he suggests the use of this technique to provide material for controlled pollination work in the greenhouse.

For greenhouse grafting, potted stock seedlings were moved in during the first or second week of January. Grafting was begun 4 or 5 weeks later, or when top growth was resumed. Humidity was maintained at a high level from time of grafting for 6 or 8 weeks. During overcast days, especially early in spring, humidity levels up to 90 percent were possible. Later, however, during bright, sunny and warm weather, when shutters were opened, daytime humidities of 35 to 60 percent were common. At the end of this period, or when the scions showed signs of growth, the humidity was gradually reduced to normal. The process occupied 4 or 5 weeks.

Early in April, the stock trees received their first pruning, which involved the removal of one side branch. The grafted trees were then moved to the lathhouse. Gradual pruning was continued for the next 3 or 4 months, or even longer, eventually forcing the scion shoot into dominance. In most cases, the grafted trees remained under partial shade during most of the hot summer months. They were moved into the open in early fall and finally outplanted later during winter or the following spring. Since these trees had been potted and their root system confined to a rather small volume, it was found necessary to water frequently after outplanting.

Grafting outdoors in the nursery was done during early spring when bud swelling occurred. Cleft, veneer, and side grafts were commonly employed. Later, "soft tissue" grafting was successfully carried on throughout the growing season.

Some form of light, unbroken shading was always employed to prevent high temperatures within the individual humidity chambers that enclosed either the entire tree or only the scion shoot. A thin muslin cloth was found satisfactory for this purpose. From 3 to 5 weeks after grafting, humidity was gradually reduced and brought to normal in 1 or 2 weeks. The grafts were placed under lathhouse shade for the greater part of the summer and moved into the open during early fall. Outplanting was carried out in the following winter or spring.

1947-1948 GRAFTING

Several unsuccessful attempts had been made previously to graft stem shoots from littleleaf-diseased shortleaf pine onto seedlings. Such grafts were desirable as a means of testing for the presence of a possible virus in diseased trees. Methods used earlier (3), such as the bark patch and approach grafts, proved inadequate when attempts were made to explore fully the virus possibility as a cause of littleleaf.

In a 1947 experiment, scion material from both littleleaf and healthy adult trees was grafted to two rows of closely spaced 5-year-old shortleaf pine seedlings in the nursery in mid-April. A total of 114 grafts were made by the cleft, side, and needle-bundle methods. The latter method was patterned after that used successfully by Mirov (6). As each graft was made, the scion shoot was enclosed in a 1-pound, translucent, wax-impregnated paper bag. A small tear in the top provided for air exchange.

Only one graft of this entire series was successful--a cleft graft made with a short shoot from a 35-year-old littleleaf shortleaf pine, which developed an excellent union. The vigorous new growth lacked any of the foliar symptoms commonly associated with littleleaf and has so continued since.

The following year, a study[1/] was initiated to determine whether exuded resin from the cut surfaces of the scion and stock hampered union. It seemed that this resinous layer might hinder the union of the respective meristematic tissues by acting as a separator. Accordingly, treatments were devised in an attempt to remove or hold back the resin from the cut surfaces until the parts had been firmly fastened.

Apparently, little consideration has been given in past work to the effect exuded resin might have upon graft union. Mirov (6) considered the resin as beneficial. He believed that it aided in sealing the openings to the cut surfaces and thus prevented drying of the tissues. Riker, et al. (8), however, believed that this exuded resin was detrimental to successful fusion in grafts of eastern white pine. They emphasized the necessity of fastening the cut surfaces of the stock and scion securely together to prevent the formation of a "resinous barrier" between them.

1/ Zak, B. Importance of resin in the grafting of shortleaf pine. M. F. thesis, Duke University. 35 pp. 1949. (Unpublished)

The stock trees used in this study were those unsuccessfully employed during the 1947 grafting. The stems had been severed about 5 inches above the root collar to produce an abundant crop of vigorous sprouts. Some of these were over 18 inches tall and up to ½ inch in diameter at their bases. Four of the best sprouts on each tree were selected for grafting and two additional ones left intact to serve as "feeders." The latter were gradually pruned away, beginning 3 weeks after the grafts were made.

Four treatments, including the control, were used to remove the resin or reduce its flow. All were applied to each stock tree; each stock sprout received a different treatment. These were:

1. Control.
2. Application of soft blotting paper to cut surfaces to remove exuded resin.
3. Base of scion shoot dipped in warm (50°C.) water for one minute to reduce viscosity of resin. Cuts were then made and resin removed as in 2. Warm water was not applied to stock stem.
4. Stock and scion tissues were cooled prior to cutting and fastening, in order to stiffen the resin and so prevent its exudation before the graft was fastened.

High humidity in the air surrounding each grafted scion stem was maintained by an individual humidity chamber made from a 5-gallon container open at both ends. This was placed over the stock tree and fastened to the ground. Moist peat moss was inserted and a glass pane placed over the top (fig. 1A). The outside of this chamber was coated with aluminum paint to reduce heat buildup within.

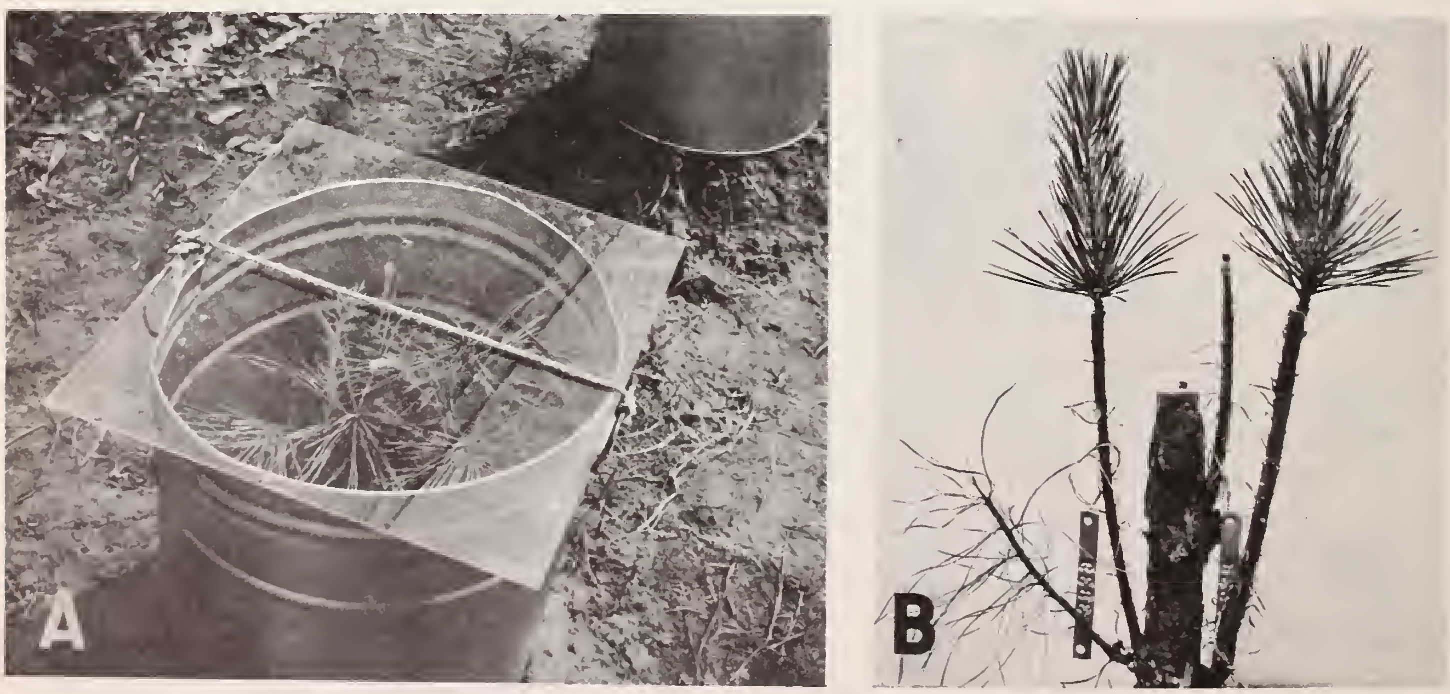

Figure 1.--Outdoor grafting of shortleaf pine. A, Portable humidity chamber installed over grafts. B, Successful grafts 2 months after grafting. Scion twigs are from littleleaf-diseased trees.

The highest success, 25 percent, was obtained in the control series (table 1). The treatments designed to remove or hold back resin failed to improve union between stock and scion. In fact, these treatments hindered union, probably by killing tissue and thus producing a "barrier" of dead cells.

Table 1.--Successful grafts by treatments and scion trees, 1948 series, shortleaf pine

Treatment	Scion trees [1/] (and age in years)						
	A(42)	B(27)	C(30)	D(38)	E(34)	F(40)	All
	Percent grafts successful [2/]						
1 (Control)	0	40	0	50	60	0	25
2	0	0	0	10	50	0	10
3	0	20	0	20	30	0	12
4	0	30	0	10	30	0	12
All	0	23	0	23	43	0	15

1/ A, B, and C healthy; D, E, and F littleleaf.
2/ Each value is based on 10 grafts made.

It is interesting to note the strong difference in success of grafting between individual scion trees. Inherited traits probably account for some of the differences. Surprisingly, scion shoots from diseased and apparently low-vigor trees grafted as well or even better than those from healthy and vigorous trees; possibly water loss through transpiration after removal of the humidity chambers was less from diseased than from healthy shoots. Littleleaf shoots, although apparently low in vigor, may be better adapted to the relatively xeric conditions imposed by grafting. The short length and poor retention of the needles as well as previous conditioning to a difficult water supply may contribute to this adaptation.

1953 GRAFTING

Upon completion of investigations on the cause of littleleaf, a program of selection and breeding was begun in an effort to develop littleleaf-resistant strains of shortleaf pine. For this program requiring asexual propagation of selected material, grafting was relied upon because of the lack of a reasonably successful rooting technique.

To improve grafting techniques, a greenhouse experiment was devised to test several types of grafting and a foliar treatment of the scion shoots. Bottle, veneer, and side grafts were tried. Superimposed upon the latter two methods was a wax treatment of the scion foliage. Half of the scion shoots in each group were dipped in a water mixture of Dowax. The other half served as control for this treatment. The purpose of this wax application was to reduce water loss from the scion tissue until sufficient union had developed.

Although the greenhouse was humidified, rather low humidities were experienced during warm sunny days as the season advanced.

Scion material from 8 selected shortleaf pines ranging in age from 33 to 81 years was grafted onto 2-year-old potted shortleaf pine stock seedlings during mid-February. When new growth began, scion material was collected from each tree, all located 150 miles distant, packed in moist sphagnum moss and stored in the refrigerator until needed.

The veneer and side grafts of the non-wax-dipped series were most successful (table 2). Seventy-six percent of the veneer grafts and 75 percent of the side grafts made good union. In general, dipping the scion shoots in Dowax adversely affected union in both methods. This treatment caused injury to the foliage; after 2 weeks, needles turned brown and water-soaked in appearance about halfway down from the tips. The wax emulsion used was apparently an older formulation containing toxic ammonium linoleate as emulsifying agent. The present Dowax is said to be free of agents injurious to plant tissue.

Table 2.--Successful grafts by treatments and scion trees, 1953 series, shortleaf pine

Scion tree		Type of graft					
No.	Age [1/]	Bottle ND [2/]	Veneer ND	Side ND	Veneer D	Side D	All
	Years	Percent grafts successful [3/]					
Z-1	35	50	90	100	70	80	78
Z-3	42	30	30	30	20	50	32
Z-7	33	0	90	70	60	40	52
Z-13	78	80	80	60	50	50	64
Z-14	81	0	100	90	40	40	54
Z-15	81	0	100	100	70	90	72
Z-16	50	0	60	70	60	20	42
Z-17	69	0	60	80	80	60	56
All		20	76	75	56	54	56

1/ Core count plus 3 years.
2/ ND means not dipped in Dowax; D means dipped.
3/ Each value is based upon 10 grafts made.

The bottle graft gave the poorest results, with only 20 percent of unions successful. This relative failure may be attributed to the post-grafting treatment. Six weeks after the grafts were made, the bottles supplying water to the bases of the scion shoots were removed. Scion stems were then severed

just below the union, since little if any water was being removed by the scion stems and bacterial contamination was feared. About one week later, many of the scion shoots commenced to brown and die. Greenhouse humidity was being gradually reduced at this time but was still rather high except on bright warm days.

1954 GRAFTING

Another group of shortleaf pines, selected for apparent resistance to littleleaf, was propagated by grafting in the greenhouse during 1954. These trees were all located in Union County, South Carolina. The techniques were similar to those employed in the 1953 work. However, only the side and veneer grafts were tried. Two-year-old potted seedlings of both shortleaf and loblolly pine served as stock trees.

Only 27 percent of the grafts were successful (table 3). Shortleaf grafted on shortleaf pine stock gave 37 percent, and shortleaf on loblolly pine stock 17 percent successful unions. Again a wide variation in grafting ability among scion trees is apparent.

Table 3.--Successful grafts of shortleaf pine scions on shortleaf and loblolly pine stocks, 1954 series

Scion tree		Stock species grafted		
No.	Age 1/	Shortleaf stock	Loblolly stock	All
	Years	Percent grafts successful 2/		
Y-1	70	27	58	43
Y-2	45	91	0	46
Y-3	45	18	7	13
Y-4	46	49	0	25
Y-5	45	94	0	47
Y-6	89	38	38	38
Y-7	43	0	7	4
Y-8	46	29	36	33
Y-9	52	20	40	30
Y-10	42	9	0	5
Y-11	43	27	0	14
All		37	17	27

1/ Core count plus 3 years.
2/ Each value is based on 45 grafts made.

Experimental work was also done with "soft tissue" or succulent grafts using techniques similar to those employed by Mergen (4) in grafting slash pine. By this method a small immature shoot is grafted into the growing tip of the stock seedling, generally by the cleft type of joining. Unlike most other forms of grafting, the shock upon the stock plant is greatly lessened since only a small section of stem is removed. Little if any pruning is required later. Union between the two parts is very rapid. Apparently, a high proportion of tissue other than the cambium may become meristematic in such young stems. The almost intact crown of the stock seedling promotes a high rate of food production and rapid tissue formation.

The soft tissue method is simple. An immature scion stem with needles only slightly developed (fig. 2A) is cleft-grafted into the growing terminal of the stock seedling. The scion shoot is cut to a length of about 1 or 2 inches and the basal end shaped into a wedge. A corresponding length of growing tip of the stock tree is removed. A short vertical cut is made into this stub and the scion stem inserted. Little care is necessary in aligning the cambial zones. The graft is then firmly wrapped with soft cotton thread (fig. 2B). Instead of being tied, the thread-wrapping is smeared on one side with a small bit of softened grafting wax to prevent unraveling. Finally, the top portion of the stock tree, including the newly-grafted scion, is enclosed in a 2-pound cellophane bag (fig. 2C). A few holes, totaling about one-half inch in area, are cut in the top for aeration. Throughout this process, care is exercised to prevent wilting of the scion shoot. The grafted seedling is then placed under a light unbroken shade. Three weeks later, the cellophane bag is partially opened to lower the humidity within. The following week, it is completely removed and the tree placed in the lathhouse. A month or two later, the grafted seedling is moved into full sunlight.

Table 4 presents the results of inter- and intra-specific grafting by this method. Scion material was taken mainly from seedlings but equally good results can be expected from older material. In most cases the union between stock and scion (figs. 2E and 2F) was excellent. Equally good results were obtained in early spring, in midsummer, and in late summer. Some of the grafting was done with no apparent adverse effects when air temperatures ranged near 35°C.

An important factor limiting this method of grafting pine is the availability of suitable scion material. On large and vigorous trees the young shoots may be too large and thick for use with this technique. Possibly this difficulty may be overcome by pruning the buds from the ends of branches to stimulate the development of several small shoots from needle bundles. It is believed this treatment may be used successfully with shortleaf and loblolly pine if pruning is done about 3 to 4 weeks before the material is needed.

Soft tissue grafting will be of considerable use to foresters in expanding the existing clones already in seed orchards. One- or two-year-old grafted trees will have sufficient crown to supply additional scion tissue. Very light pruning or even tip moth damage of these trees will encourage the development of many small shoots. Each tree should supply 10 or 15 scion stems without harm.

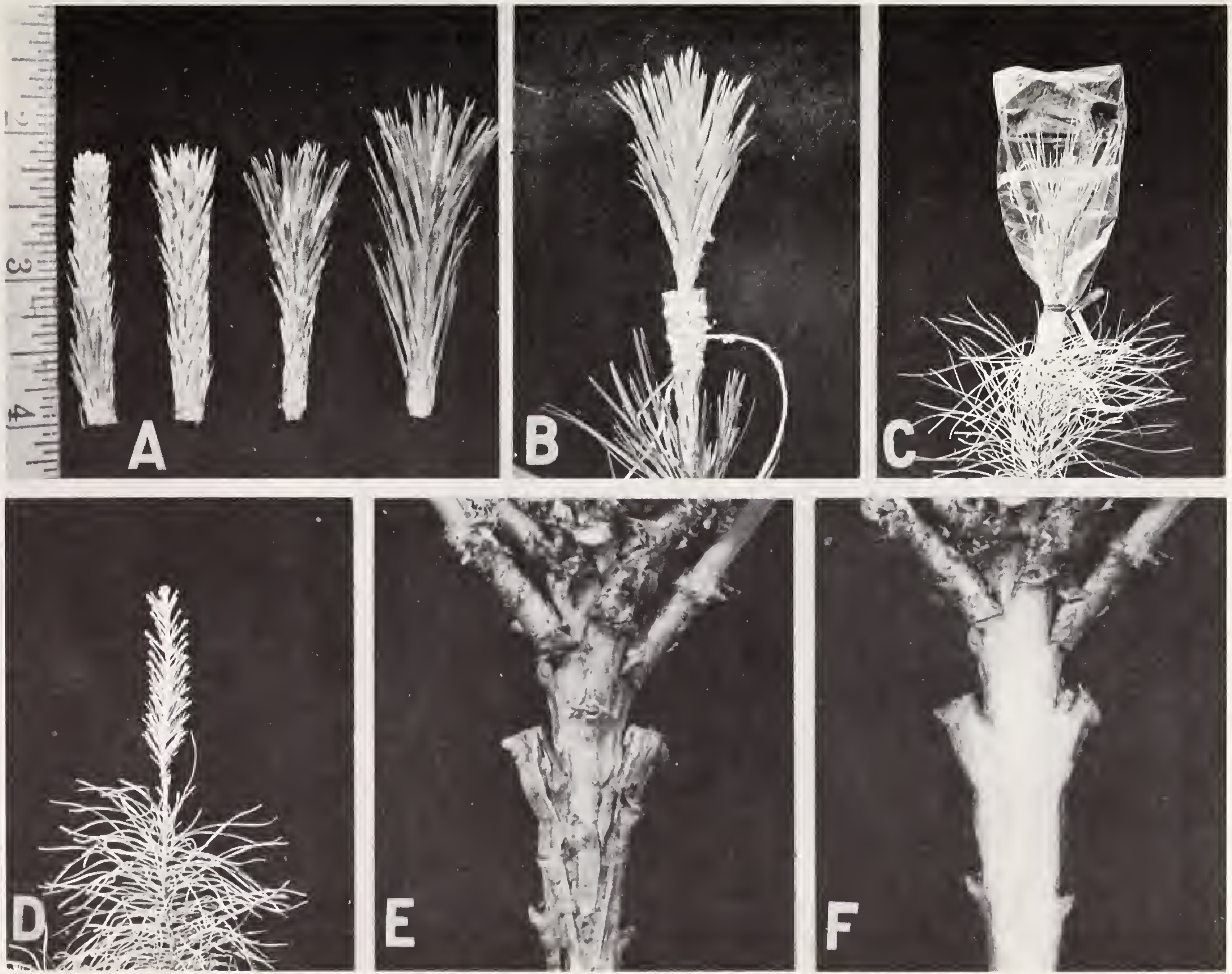

Figure 2.--Soft tissue grafting of pine. A, Scion shoots suitable for use with this method. B, Completed graft. C, Graft enclosed in cellophane bag to maintain high relative humidity in air surrounding scion shoot. D, Graft of shortleaf on Virginia pine stock after 2 months. E, Close-up view of graft union. F, Graft union of E cut open to show excellent fusion of scion and stock.

Table 4.--Soft tissue grafting results, 1954 series

Scion tree		Stock tree		Grafts	
Species	Age	Species	Age	Made	Successful
	Years		Years	Number	Number
Shortleaf	3-46	Shortleaf	2	25	18
Loblolly	2	Loblolly	2	5	4
Loblolly	2	Shortleaf	2	4	2
Shortleaf	2	Loblolly	2	8	5
Slash	2	Loblolly	2	4	2
Slash	2	Shortleaf	2	3	2
Loblolly	2	Virginia	2	3	1
Shortleaf	2	Virginia	2	3	3
Eastern white	30	Virginia	2	5	2

GENERAL DISCUSSION

Success or failure of plant grafting is largely a matter of water relationships in the scion stem and later in the scion-stock combination. After the scion shoot has been severed from the parent plant and lacks a significant source of water, loss of water by transpiration may continue at a high rate and lead to dehydration of the tissue. The problem then becomes one of water conservation until adequate connective xylem is formed between stock and scion. Once a good union had developed, sufficient water can pass from stock to scion to replace that lost by transpiration.

Transpiration of water from the scion stem is dependent on environmental conditions and on the character of the shoot itself. Full sunlight, high air temperature, and low relative humidity in the air surrounding the scion all contribute toward a high rate of transpiration. Therefore, when grafting, it is desirable to provide some form of shading and to maintain high relative humidity of the air. Shading is used only to reduce heat build-up within the greenhouse or humidity chamber. Adequate light is desirable for synthesis of food to promote rapid tissue formation.

The type and size of scion shoots must also be considered. Succulent material, as used in soft tissue grafting, is far more sensitive to conditions of high transpiration than is mature and hardened tissue. The writer found that tender shoots open-grafted in the greenhouse readily wilted without recovery after 1 or 2 days. Even under humidification, levels as low as 35

percent relative humidity were encountered on bright warm days. No early wilting occurred when the shoot was enclosed in a cellophane bag (fig. 2C) together with some of the stock foliage. Humidity within was almost continuously near saturation.

When mature stems are being grafted, the portion above the union should be relatively short in length. Less surface is thus exposed to transpiration. Often, half the length of the remaining needles is sheared off to further reduce water loss from the scion stem. A suitable nontoxic wax emulsion applied over the foliage might be useful in lowering water loss.

The extent and rapidity of union between stock and scion are also important factors in successful grafting. The greater the area in contact between the two parts, the better the chance of success. It is important, therefore, to match the diameters of the two stems so that cambial zones on both sides can be aligned. Stems of unequal size may be used, however, by carefully regulating the depth of cut so that width across the cut surface of each is approximately the same. Accurate alignment of the stems is necessary for success. Rapid knitting of the union is promoted by favorable growing conditions and by the use of vigorous stock.

Finally, careful consideration should be given to the manner of pruning the stock tree after grafting, in order to force the scion shoot into dominance. Little if any pruning is necessary with the soft tissue grafts; but when mature stems are grafted by the bottle, side, or veneer methods, pruning of the stock tree is desirable. Eventually all stock tissue above the union is removed, leaving only the scion to grow.

Too early and too rapid pruning often result in graft failures. Quick removal of the stock crown after grafting causes too great a reduction in the stem diameter growth, and consequently delays or even prevents the development of a sound union. Pruning, therefore, should be gradual and should extend throughout most of the growing season. Actually, each tree should be treated individually according to its size and according to the rate of development of the scion shoot. The first pruning should always be very light. Removal of a small side branch may be sufficient. Then as the volume of scion foliage increases, more and more of the stock crown can be removed until only that of the scion remains.

SUMMARY

The grafting of shortleaf and other pine species in connection with investigations of the littleleaf disease is described and the factors affecting successful grafting are discussed.

The work reported is a continuation of that described by Jackson and Zak (3). Studies from 1947 to 1954 are included. Grafting techniques were first employed to test a virus hypothesis for littleleaf and later as tools in the selection and breeding of littleleaf-resistant pine.

Various grafting methods such as the cleft, bottle, side, veneer, and "soft tissue" were used, both in a humidified greenhouse and outdoors in the nursery. Scion material from trees ranging in age from 2 to 89 years was successfully grafted.

In a 1947 experiment, only 1 graft of 114 was successful. The next year, a study was carried out to test the effect of exuded resin upon union between stock and scion. Treatments used to remove or retard resin flow proved injurious to exposed tissues and caused a reduction in the number of successful unions. Successes up to 60 percent of the grafts were obtained for individual scion trees and treatments.

Bottle, side, and veneer grafts were tested in another study using shortleaf pine. A Dowax dip-treatment of the scion shoots was superimposed upon the side and veneer methods. The undipped series of the side and veneer grafts gave about 75 percent success and the wax-dipped series only 55 percent. The lower success of the latter is ascribed to toxicity of the emulsifying agent contained in an older formulation of the wax used. Bottle grafts were only 20 percent successful.

Both shortleaf and loblolly pine stock seedlings were used in a 1954 trial. Scion material came from selected shortleaf pines. Shortleaf scions on shortleaf pine stock gave 37 percent and shortleaf on loblolly pine gave 17 percent success. As in previous work, individual scion trees varied greatly in their ability to form successful unions.

The soft tissue method of grafting is described and the results of various inter- and intra-specific grafting are given. The method shows great promise for use in tree improvement and genetics investigations. It may prove especially valuable for expanding existing clones in seed orchards.

The principal factors affecting successful union in pine grafting are discussed. Water relations of the severed scion stem and later of the stock-scion combination are deemed of prime importance.

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